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Use of streak camera for time-resolved photon counting fluorimetry

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Abstract. The use of a conventional streak camera for subnanosecond time-resolved fluorimetry is extended to the single-photon counting regime by utilizing intensified readout of the phosphor. The system achieves the usual key advantages of photon counting, namely single-photon sensitivity, large dynamic range and shot noise limited statistics, but also permits measurements of lower repetition rate and faster time response phenomena, in regimes inaccessible to microchannel plate photomultiplier instrumentation. Pile-up and problems associated with the slow decay and non-uniformity of the response and readout of the streak camera phosphor are discussed.

1. Introduction

For the ill-conditioned problem of resolving multi-component fluorescence decay profiles, time-correlated single-photon counting (TCSPC) yields high-quality data over a large dynamic range and with accurately known errors derived from the Poisson photon statistics [1]. The fastest temporal resolution available, using microchannel plate photomultipliers, is at best 20 to 50 ps, as measured by the full-width-at-half-maximum (FWHM) of the prompt function [2]. The photomultiplier count rate must be kept less than the laser excitation rate to avoid pile-up and distortion of the decay profile [3].

By contrast, a streak camera with internal gain sufficient for detecting single photons allows resolution of many individual photons after each laser excitation pulse, thus allowing photon counting measurements at lower laser repetition rates. In addition, the overall time resolution can be an order of magnitude faster than that obtained by the fastest microchannel plate photomultipliers.

Although a streak camera with a high-gain double-stage internal intensifier intended for single photon counting is now commercially available [4, 5], we have demonstrated that an equally efficient method for detecting single photons is to use moderate gain on the single-stage internal intensifier of a regular streak camera, together with low-noise high-gain intensified readout of the phosphor [6]. In this paper, we give details of the implementation of this system for accumulating subnanosecond decay profiles, with a sample excitation repetition rate of only 10 Hz. Standard data analysis techniques for convolution and non-linear curve fitting [1, 7] are then applied to the acquired plots of number of photon counts against time in order to resolve multiple exponential components from the decay profiles.

We discuss problems, such as the slow decay of the streak camera phosphor, which can lead to multiple counting of the same photon, and pile-up, which occurs at high photon detection rates. We test the capabilities of the system for resolving multiple decay components using solutions of organic dyes with known lifetimes. We describe the use of the system for applications which cannot be addressed by conventional TCSPC, and conclude with a brief discussion of the time resolution attainable with this system.

2. Experimental configuration

Figure 1 is a block diagram of the configuration of commercially available components used to achieve time correlated photon counting at low repetition rates. In most experiments, a Lambda Physik FL4000 excimer laser, which delivers 4 mJ, 308 nm, 35 ps pulses at 10 Hz is used for sample excitation. Emission from the sample is imaged onto the streak camera slit through appropriate optical filters (not shown in the figure).

With each laser pulse, up to about a hundred individual photoelectrons from the streak camera photocathode are resolved as the streak camera sweeps. Each photoelectron experiences a moderate gain of about 2500 due to the internal microchannel plate (MCP) intensifier and causes a bright spot on the streak camera phosphor. For high-gain low-noise readout of the streak camera phosphor we use a Hamamatsu C2400-20 camera, which consists of a Peltier-cooled photocathode, MCP intensifier (operated at low gain) and CCD camera.

The video signal from this is processed by the Hamamatsu Argus 100 camera control and image processing unit. Each video frame is digitized into an 8 bit

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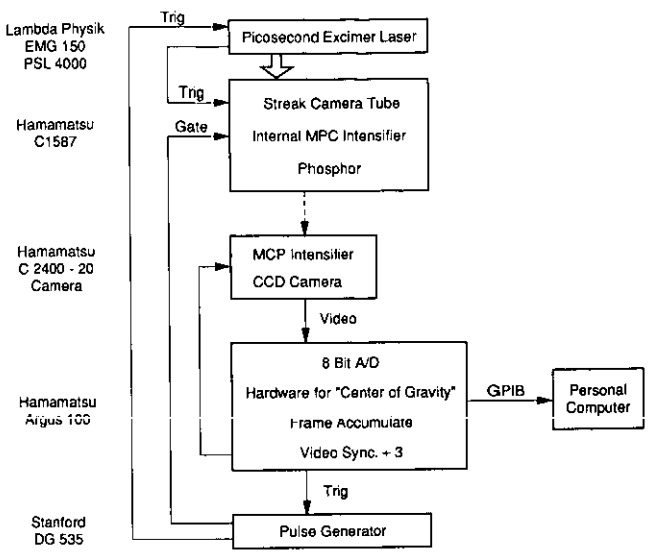

Figure 1. Configuration of instruments used to achieve time correlated photon counting with a streak camera and 10 Hz picosecond excimer laser.

512 × 480 pixel format and processed on-line by specialized hardware on the Argus 100 unit, which yields a single count at the centre of gravity (COG) of each photon image. The counts are then accumulated in a frame memory. In this way true photon counting is achieved; the non-linearity due to saturation of gain and the statistical nature of the gain process of the streak camera and readout camera are removed. There is also an enhancement in spatial resolution which corresponds to an enhancement in temporal resolution. This is analogous to the enhancement in time resolution in conventional TCSPC, obtained by using a constant fraction discriminator to define better the arrival time of photons.

3. Comparison with single-photon counting streak tube

There are two major differences between the single photon counting streak camera and the regular ('universal') streak camera made by Hamamatsu.

Firstly, the photon-counting tube is made with a smaller photocathode in order to reduce dark counts. However, if the photocathode and internal intensifier are gated on for $<1\ \mu s$ at a repetition rate of 10 Hz, dark noise is quite negligible. Even for a gating duty cycle of about 10% on time, we have found that dark counts are tolerable, and may be accounted for in the analysis of the decay profiles as a constant background level.

Secondly, the photon counting tube has a double-stage, internal, microchannel plate intensifier which gives considerably more gain and a well peaked distribution of photon image intensities. The regular streak camera tube gives photon images at the phosphor which are not as bright and which have a falling exponential distribution of intensities [4].

However, our measurements of the overall quantum efficiency, given in section 5, indicate that photon images are counted with close to 100% efficiency, despite the exponential distribution of small intensities. Moreover, since the COG hardware of the Argus yields a single count for each photon image, so that all photoelectrons from the streak camera photocathode give rise to exactly one count, the pulse height distribution of the single-photon images is not of primary concern.

4. Synchronization and phosphor decay

The decay time of the streak camera phosphor is perhaps of more importance than the gain or dark noise of the tube. It must be longer than the actual time required to read the whole screen, otherwise part of the screen will decay before it is read. However, if it is longer than the time *between* complete readouts of the phosphor, some photons will be multiply counted. Ideally, a photon counting streak camera would use a fast decaying phosphor and a synchronized integrating readout camera, such as a CCD.

In our experiments, the decay time of the phosphor is observed to be comparable to a video frame period (1/30 s), so that single photon images on the streak camera phosphor take between one and three video frame intervals to completely decay. In order to avoid counting the same single photon more than once, it is necessary to use only one phosphor readout per laser pulse.

This is accomplished by using software to reduce the rate of the Argus 100 camera controller internal video-synchronization signal by a factor of three, so that only ten video frames per second are read. The resultant 10 Hz signal (obtained internally with a small modification to the Argus unit) is then used as a reference for triggering the laser and gating the streak camera, as shown in figure 1.

5. Pile-up and quantum efficiency

In conventional TCSPC the count rate is usually kept below 0.01 times the laser excitation rate because if more than one photon is detected with each laser pulse, the timing electronics will respond to only the first photon and the decay profile will become biased towards early times [3]. By contrast, in this form of time correlated photon counting, the streak camera may detect about 100 photons with each laser pulse. If the number of photons detected in each video frame were much larger than this, the images of individual photons would no longer be resolved and two or more photons would give rise to only one count as shown in figure 2. This undercounting of photons would be more likely to occur at times when the photon flux is larger, at the beginning of the luminescence decay, so that the decay profile would become biased towards later times. This form of pile-up can also occur in conventional TCSPC using

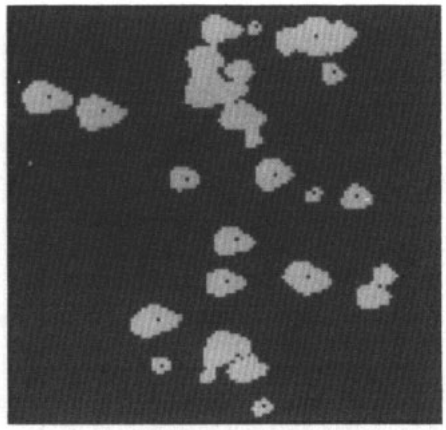

Figure 2. A 100 × 100 pixel portion of the streak camera phosphor readout, showing the results of implementing the 'centre of gravity' processing on an image. This figure also shows the pile-up or undercounting of photons which results if the incident photon flux is too high.

photomultipliers, but pile-up in the timing electronics (at the time-to-amplitude converter) is usually predominant [8].

In order to demonstrate that the images of the streak camera phosphor are due to single photoelectrons and are not merely multiphoton pile-up events, a careful measurement was made of the overall quantum efficiency of detection.

The beam from a HeNe laser was expanded using a telescope and attenuated using calibrated neutral density filters to a photon flux of 7.98×10^{13} photons $cm^{-2}\,s^{-1}$ which corresponds to an average of 1760 photons per 6.23 nanosecond sweep through the 3.54 mm × 0.1 mm slit. The actual number of photon images collected after 436 sweeps was 20 536, corresponding to an average of 47.1 per sweep. Since the transmission of the non-coated streak camera input optics is 0.65 the overall quantum efficiency (QE) of photon detection is 0.041. A similar measurement using nanosecond 308 nm pulses gives a QE of 0.16. These values agree well with the specified QE of the multi-alkali/UV-glass streak camera photocathode. Moreover, such overall efficiency measurements indicate that the readout of photon images at the streak camera phosphor is close to 100 per cent efficient.

Since the area of a photon image at the phosphor is about 10 pixels × 10 pixels, there are about 51 × 48 non-overlapping space-time locations on the 512 × 486 screen for the 47.1 photon images to fall on. Assuming the photons are randomly distributed in space and time, the Poisson probability of having more than one photon in the same location is 1.83×10^{-4} which is <1% of the probability for having one photon. Thus we may conclude that multi-photon pile-up events account for <1% of the photon images.

Provided that the photons are randomly distributed, the incident photon flux may be increased so as to give up to 100 photon images per sweep with less than 2% pile-up. When measuring the prompt function or very fast decay components, the detection rate must be kept below about 10 photons/sweep in order to avoid excessive pile-up.

6. Calibration and data reduction

The accumulated photon counts are transferred from the frame memory of the Argus 100 to a personal computer via GPIB and are summed over the spatial streak camera slit dimension to yield a plot of photon counts against temporal channel. The time axis is calibrated in the usual way by using the pulsed laser and beamsplitters to give a series of laser pulses of known temporal separation. For the fastest streak camera sweep rate (0.3 ns/15 mm) and 1:1 imaging of the streak camera phosphor we obtain a temporal calibration of 12.1 ps per channel.

To compensate for the non-uniformity of the streak camera slit transmission and the response of the photocathode, intensifiers, phosphor and CCD camera, a calibration curve is collected by uniformly illuminating the streak camera slit with a HeNe laser or a light bulb. As shown in figure 3, the overall response clearly falls off towards the edges, resulting in a non-linearity of approximately ±10%. However, this non-linearity is well reproducible, and so may be removed by dividing all experimental profiles by the calibration curve, normalized to an average value of unity. The error in the calibration curve is largely due to shot noise, which is minimized by collecting a calibration curve with good statistics. However, these errors add further noise to the normalized data which must be taken into account in the subsequent curve fitting routine as explained below.

It has been our experience that conventional TCSPC is subject to a similar non-linearity, particularly when

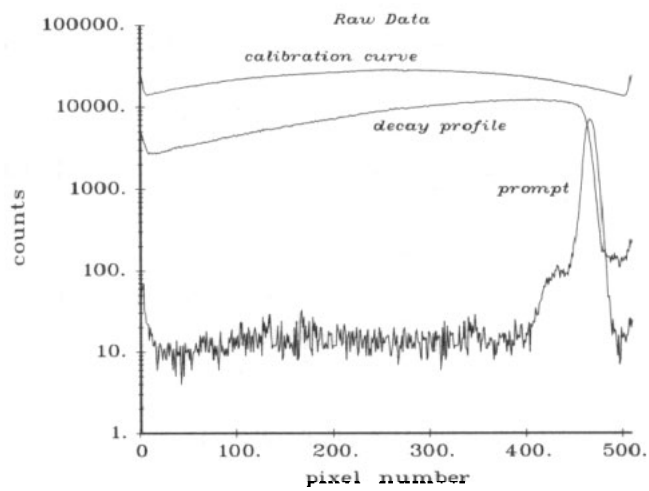

Figure 3. The calibration curve, used to correct for an overall non-linearity of response with time, was obtained by collecting light from a steady source. The uncorrected data for one of the fluorescence decay profiles and for the prompt function are also shown on this semilogarithmic plot. The channel number corresponds to time increasing from right to left (12.1 ps per channel).

recording decay profiles over fast time ranges. Although time-to-amplitude converters typically specify an integral non-linearity of 0.1%, radio frequency pick-up and oscillations in the wide band amplifiers and other electronics usually limits the overall integral non-linearity. For example, Davis *et al* [7] with a Hewlett Packard 8447F amplifier, Tennelec TC454 constant fraction discriminator and TC863 time-to-amplitude converter and a time range of 2 to 14 ns, obtained a non-linearity of at best ±5%, thereby necessitating data recalibration for accurate lifetime measurements. (When performing TCSPC one should always check the overall non-linearity.)

As a test of the capabilities of the streak camera system for accurately measuring and resolving exponential decay components, fluorescence lifetimes of known organic dyes were measured using highly attenuated pulses from the excimer laser. Figure 3 shows the raw decay curve from a 10^{-6} M aqueous solution of Rhodamine 6G, the calibration curve, and the prompt function obtained by filling the cuvette with water and replacing the 308 nm blocking filter with a neutral density filter. Figure 4(*a*) shows the recalibrated prompt function, a recalibrated decay profile, in this case from a 10^{-6} M solution of DODCI dye, and a curve fit of the decay profile. A plot of the weighted residues is shown in figure 4(*b*).

The curve fit was obtained using a modified version of a program which was originally developed for analysing conventional TCSPC data, by recursive convolution and weighted non-linear curve fitting [7]. That is, the fitting function at channel n, $F(n)$, is taken to be the convolution of the recalibrated prompt curve (with background subtracted) $i(n)$, with a sum of one to three exponential decays, with lifetimes τ_j and pre-exponential factors c_j, a temporal shift parameter s, and a constant background term b:

$$F(n)=\sum_j q_j(n+s-\tfrac{1}{2})+b.$$

Recursive convolution [1] gives the fitting function components as

$$\tilde{q_j}(n)=e_j[e_jq_j(n-1)+c_ji(n)]$$

with $e_j=\exp(-1/2\tau_j)$, and the derivatives of the fitting function as

$$\partial F(n)/\partial c_j=q_j(n)/c_j$$

$$\partial F(n)/\partial e_j=r_j(n)=e_j[2q_j(n-1)+e_jr_j(n-1)]+c_ji(n)$$

$$\partial F(n)/\partial b=1$$

and

$$\partial F(n)/\partial s=F(n)-F(n-1).$$

As is usually the case with recursive convolution and curve fitting, errors in the prompt curve which propagate into the fitting function are ignored.

When the decay profile is recalibrated by dividing by the normalized calibration file, the weights to be used by the non-linear curve fitting routine [9] are also recalculated. The weight applied to each point should

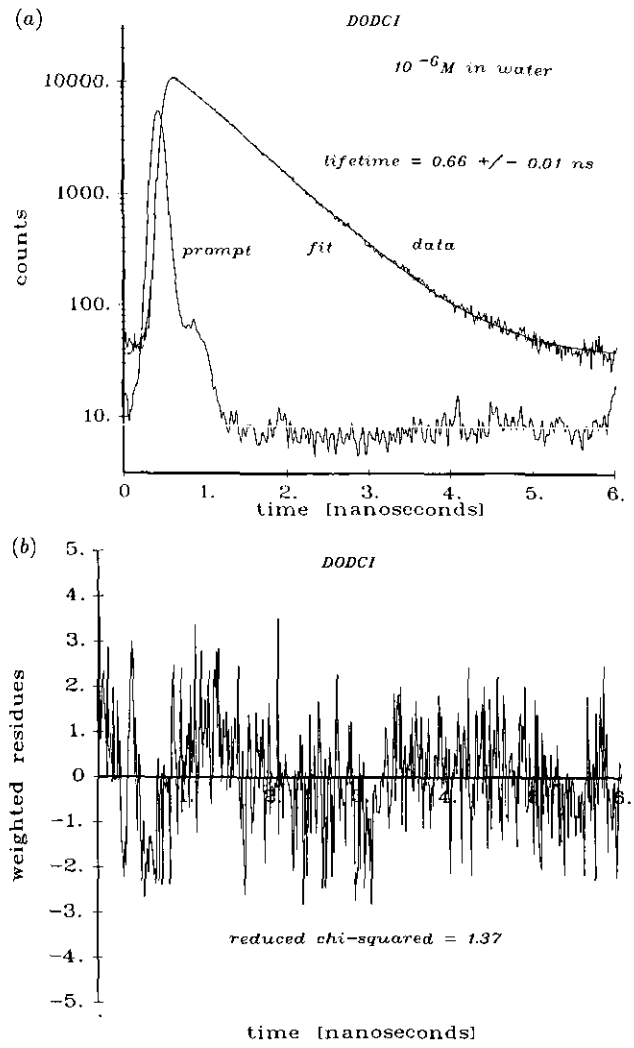

Figure 4. (*a*) The recalibrated decay profile from DODCI dye solution excited by the attenuated picosecond excimer laser pulses yields a single exponential lifetime of 0.66 ns. (*b*) The weighted residues for the fit are evenly distributed about zero, although $\chi^2=1.37$, possibly indicating the presence of other random errors.

be the reciprocal of the variance at that point and, whereas for simple Poisson errors, the variance at each point is equal to the value at that point, in the case of the recalibrated decay profile the variance at each point is simply the sum of the raw value at that point and the value of the calibration curve at that point.

The plot of the weighted residues shown in figure 4(*b*) is well distributed about zero, indicating that an exponential fit is quite adequate. However, the value of the reduced chi-squared ($\chi^2=1.37$) is greater than unity, indicating the probable existence of additional random but uniform errors which have not been included in the weights.

The data of figure 4(*a*) yield a lifetime of 0.66 ± 0.01 ns in exact agreement with that found by conventional TCSPC using a 76 MHz mode-locked frequency-doubled Nd:YAG laser for sample excitation. In a similar way, the profile from figure 3 yields a lifetime of 3.62 ± 0.04 ns for 10^{-6} M aqueous Rhodamine 6G, in accord with a

previously determined value of 3.61 ± 0.01 ns. The errors in the parameters are estimated by the curve fitting program from the partial derivatives of the reduced chi-squared.

The capability of resolving multiple exponential components in a decay profile using these techniques has been previously established using synthetic data but was re-established here by measuring decay profiles of mixtures of the two dyes DODCI and Rhodamine 6G. Lifetimes and proportions were within the error bounds of those expected although the estimated errors are quite large if one component dominates. For example, for a 20:1 mixture of 10^{-6} M DODCI and 10^{-6} M Rhodamine 6G, analysis of the decay profile gives $97 \pm 1\%$ of 0.67 ± 0.01 ns component and $3 \pm 1\%$ of 3.29 ± 0.51 ns component ($\chi^2 = 1.83$).

7. Applications and discussion

Without attenuation, the pulses from the picosecond excimer laser used in this work have sufficient energy to give optical breakdown easily when focused in air or at surfaces. In general, laser systems which operate at repetition rates around 10 Hz can give fluences several orders of magnitude greater than the kilohertz and megahertz repetition rate laser systems required for conducting conventional TCSPC. Thus there are several applications of this technique for time-resolved photon counting which cannot be addressed by conventional TCSPC.

One such application is the investigation of the luminescence decay of picosecond laser induced plasmas. Figure 5(*a*) shows the decay curve from a laser ablation plasma formed by focusing 1.0 mJ 35 ps excimer laser pulses onto an aluminium target in air. The luminescence was collected through a 360 nm long pass filter which completely blocks the scattered excitation radiation at 308 nm. The decay profile clearly shows more than one exponential component (a one exponential fit gives $\chi^2 = 12.92$) and a two-component fit yields lifetimes of 0.31 ± 0.04 ns and 9.77 ± 0.03 ns. However, in this case $\chi^2 = 2.92$ and the weighted residues, shown in figure 5(*b*), exhibit large deviations near the prompt and a non-random trend which suggests the presence of a third component.

Another possible area of application is the interaction of ultra-high laser fluences (up to 10^{19} W cm^{-2}) with atomic systems. There has been considerable recent progress in this area [10] and experimental capabilities may be enhanced by applying photon counting detection.

Applications are not limited to low repetition rate laser excitation. We have used a frequency doubled 76 MHz mode-locked laser system to induce very weak, fast decaying, ultraviolet fluorescence in biological DNA samples [11]. Here, we use a synchroscan time base plug-in for the streak camera, which allows the streak camera to sweep synchronously with the excitation and photons to be collected with each laser pulse. However, in order to avoid pile-up, no more than about 100

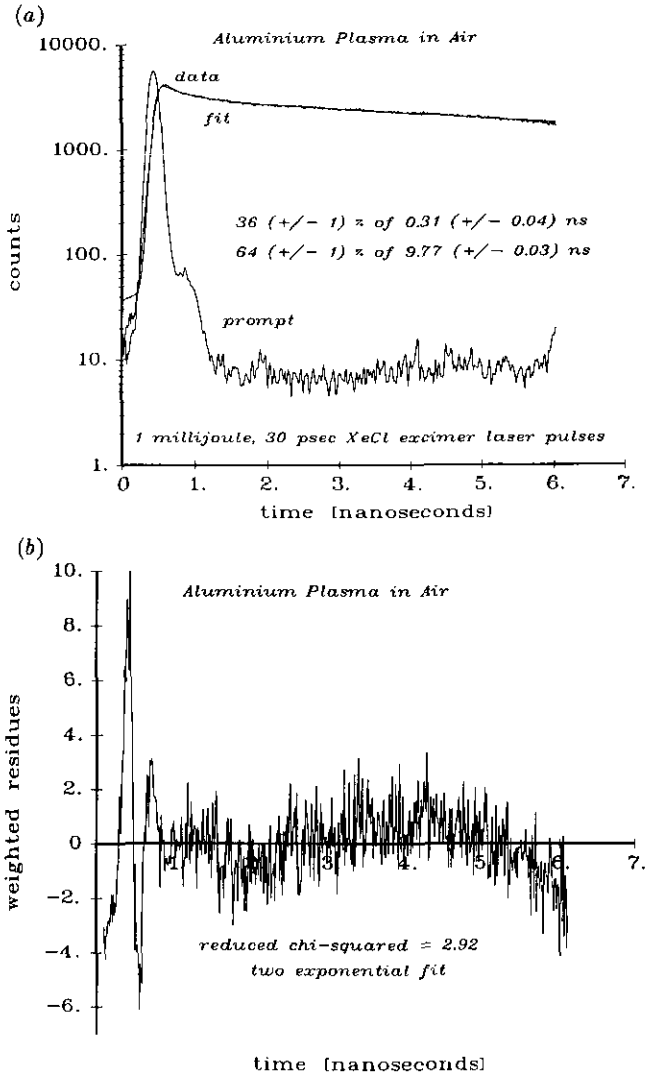

Figure 5. (*a*) The luminescence decay profile from a laser ablation plasma formed by focusing 1.0 mJ, 35 ps pulses at 308 nm onto an aluminium target in air shows components at 0.31 and 9.77 ns. (*b*) The plot of the weighted residues in this case exhibits large deviations near the prompt and a non-random trend which suggests the presence of a third component.

photons may be collected over each 1/30 s video frame period, corresponding to an average of only 4×10^{-5} photons per laser pulse. In addition, if multiple counting of the same photon is to be avoided, the streak camera phosphor should only be read at 10 Hz and the streak camera should be gated off for $\frac{2}{3}$ of the time. Thus, data acquisition is quite inefficient, but the time resolution is faster than that achievable using conventional TCSPC. A prompt function with 14 ps FWHM and 60 ps width at 0.01 maximum is readily obtainable.

For experiments using the low repetition rate excimer laser for sample excitation, the high-speed streak camera time base was used. This has a 2 ps specified time resolution for single-shot analogue measurements but for photon counting the time resolution is limited by the jitter of the signal used to trigger the streak camera, which in this work is typically 50 ps FWHM. However, it

should be possible to reduce this to result in an overall time response of about 2 ps by using a photoconductive switch to trigger the streak camera sweep [12]. Sub-picosecond FWHM prompts may in the future be achievable using these techniques with the new generation of femtosecond streak cameras.

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